

WHAT IS

BIOREMEDIATION?

INTRODUCTION

Bioremediation technology uses microorganisms to reduce, eliminate, or contain contaminants. It is not a new technology, however. Composting, sewage treatment, and certain types of fermentation have been practiced by humankind since the beginning of recorded history, and all of these utilize microbial processes. Evidence of kitchen middens and compost piles dates back to 6000 B.C. And the more “modern” use of bioremediation began over 100 years ago with the opening of the first biological sewage treatment plant in Sussex, UK, in 1891. Yet the word “bioremediation” is fairly new. Its first appearance in peer-reviewed scientific literature was in 1987 (Hazen, 1997).

The use of this technology is gaining popularity. The last ten years have seen an increase in the types of contaminants to which bioremediation is being applied, including solvents, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs). Now, microbial processes are beginning to be used in the clean up of radioactive and metallic contaminants, the most recalcitrant components of hazardous waste, and the two most often found components of mixed waste at DOE sites, as discussed in Section I.

This primer looks at the possibilities for in situ bioremediation of these types of contaminants.¹ Featured are eight elements that constitute some of the most prevalent metals and radionuclides found in DOE waste: cesium, chromium, lead, mercury, plutonium, uranium,

strontium, and technetium. All are metallic elements and very toxic. In addition, cesium, plutonium, strontium, technetium, and uranium are extremely radioactive. (See the inside front cover for a periodic table that highlights these elements.) In situ bioremediation of these contaminants, particularly in contaminant mixtures, is not yet in widespread use. However, successful in situ applications of bioremediation to petroleum products and chlorinated solvents are a resource from which the scientific community can draw. The accomplishments in these areas have led scientists and engineers to be optimistic about applying this technology to the mixtures of metals and radionuclides that are found at some of the most contaminated DOE sites.²

Many remediation technologies exist to treat hazardous waste. One of the most common has been pump and treat (extraction and then treatment by various processes). Pump and treat is often applied in the remediation of industrial solvents such as trichloroethylene (TCE), which is used to degrease metals (including nuclear target elements and computer components), dry-clean clothes, and even decaffeinate coffee.

Extraction processes do have some major disadvantages. Subsurface sediment and rock formations are heterogeneous. This lack of uniformity can cause uneven flow patterns. So it can take a long time to flush contamination out of areas where water flow is slow. Many contaminants also tend to adsorb (stick) to mineral

1. This contamination often exists in the form of contaminant plumes. See the feature on page 14 for more information on how the subsurface is structured and how these plumes move.

2. Although organic components are a part of mixed waste at DOE sites, they are not the focus of this primer. However, certain organic compounds play a central role in determining metal and radionuclide bioremediation strategy. The synthetic chelators EDTA (ethylenediaminetetraacetic acid) and NTA (nitrilotriacetic acid) were commonly used as cleaning agents during industrial processing of nuclear fuels at DOE and have formed stable, soluble complexes with certain heavy metals in the subsurface.

surfaces of clays or to organic materials. This can slow extraction, and it often takes decades before enough contaminant is removed to make a site safe. Also, bringing the contaminants up to the surface can increase health and safety risks for cleanup workers and the public.

Bioremediation is an alternative to traditional remediating technologies, such as landfilling or incineration. It works by either transforming or degrading contaminants to nonhazardous or less hazardous chemicals. These processes are called, respectively, biotransformation and biodegradation.

Biotransformation is any alteration of the molecular or atomic structure of a compound by microorganisms. Biodegradation is the breaking down of organic substances by microorganisms into smaller organic or inorganic components. Mineralization is the complete biodegradation of an organic contaminant into inorganic constituents such as carbon dioxide and water. Under anaerobic conditions, the ultimate product of biodegradation may be methane. This complete degradation of a compound is the end result of numerous biodegradation steps. These transforming and degrading processes occur as a result of microorganisms using the contaminants as a source of nutrients or energy, changing them through various metabolic reactions.

Unfortunately, metals and radionuclides cannot be biodegraded. However, microorganisms can interact with these contaminants and transform them from one chemical form to another by changing their oxidation state.³ In some cases, the solubility of the altered species increases, increasing the mobility of the contaminant and allowing it to more easily be flushed from the environment. In other cases, the opposite will occur, and the contaminant will be immobilized in situ, thus reducing the risk to humans and the environment. Both kinds of transformations present opportunities for bioremediation of metals and radionuclides — either to lock them in place or to accelerate their removal.

Although bacteria are usually the agents in most types of bioremediation, fungi and algae also can transform and degrade contaminants. Bioremediation depends on the presence of the appropriate microorganisms in the correct amounts and combinations and on the appropriate environmental conditions. Optimum environments for growth of microbes typically consist of temperatures ranging between 15 and 45°C;⁴ pH values between 5.5 and 8.5; and nutrient ratios (C:N:P) of 120:10:1. Atmospheric composition and water content may also influence microbial growth and activity. In addition, the contaminants must be in close enough proximity to the microbes and in a form that the microbes can utilize.

WHICH BIOREMEDIATION TECHNOLOGY SHOULD BE USED?

Webster's Dictionary defines *in situ* as "in place; in the natural or original position or place." *In situ* bioremediation refers to below-ground methods applied at the site of contamination. Webster's defines *ex situ* as "in a position or location other than the natural or original one," but this usually refers to above-ground bioremediation, where the sediment or water has been extracted from the subsurface.

There are a number of *ex situ* and *in situ* bioremediation methods currently available. *Ex situ* methods have been around longer and are better understood; they are easier to contain and control. However, *in situ* bioremediation has several advantages over *ex situ* techniques. It offers a way of treating contaminants that are widely dispersed in the environment, present in dilute concentrations, or are otherwise inaccessible. It is more

3. Microorganisms can do much more than alter oxidation state. They are also capable of influencing contaminant behavior in other ways. Examples include changing the acidity of the system in the immediate vicinity of the contaminant and alteration of the form of organic compounds that influence radionuclide and metal mobility. Although important, these factors are not the main focus of this primer.

4. Although recently it has been discovered that petroleum bioremediation in the Arctic and Antarctic can occur at nearly the same rates at near zero degree temperatures (°C) as are seen in more temperate climates.

cost effective than ex situ techniques because no pumping or excavation is required. Also, in situ bioremediation may be less hazardous, as there is no exposure to the contaminant during treatment. This is a consideration because of the mixing of metals and radionuclides with organic contaminants at DOE sites. This mixing has resulted in modification of the contaminants' transport and toxicity properties, which often imposes an increased health risk.

Next is a brief overview of several existing bioremediation strategies. As Figure 2.1 demonstrates, these methodologies are not mutually exclusive and, depending on the type of contaminant problem, can be used in combination with one another and/or with more traditional remediation techniques.

Biostimulation and Bioaugmentation

These two bioremediation techniques can be used together or separately. They can occur either above ground (in stirred tanks called bioreactors) or below ground. Biostimulation is the addition of nutrients, oxygen, or other electron donors and acceptors to increase the number or activity of naturally occurring microorganisms available for bioremediation. These components can be added

in either liquid (soil washing) or gas (soil venting) form. Biosparging is a type of soil venting where air or other gases are injected below the ground into saturated sediments.

All microorganisms need carbon. Carbon usually comes from an organic source (e.g., glucose or methane), but also can be provided in dissolved inorganic forms such as carbon dioxide (CO_2). Sometimes the contaminant is a carbon source, as in the case of gasoline contaminants such as benzene, toluene, and xylene. Waste products from plants and other microorganisms also can provide carbon. Some of the other most common microbial nutrients are nitrogen, phosphorus, and sulfur. Nitrogen and phosphorus are found both organically and inorganically, and are often present in soil, sediments, and groundwater.

Bioaugmentation is the addition of microorganisms that can biotransform or biodegrade a particular contaminant. To date, bioaugmentation has not been consistently effective in a subsurface environment. However, bioremediation can be enhanced by the continuous addition of microorganisms to a bioreactor for the above-ground treatment of contaminated groundwater. Organisms

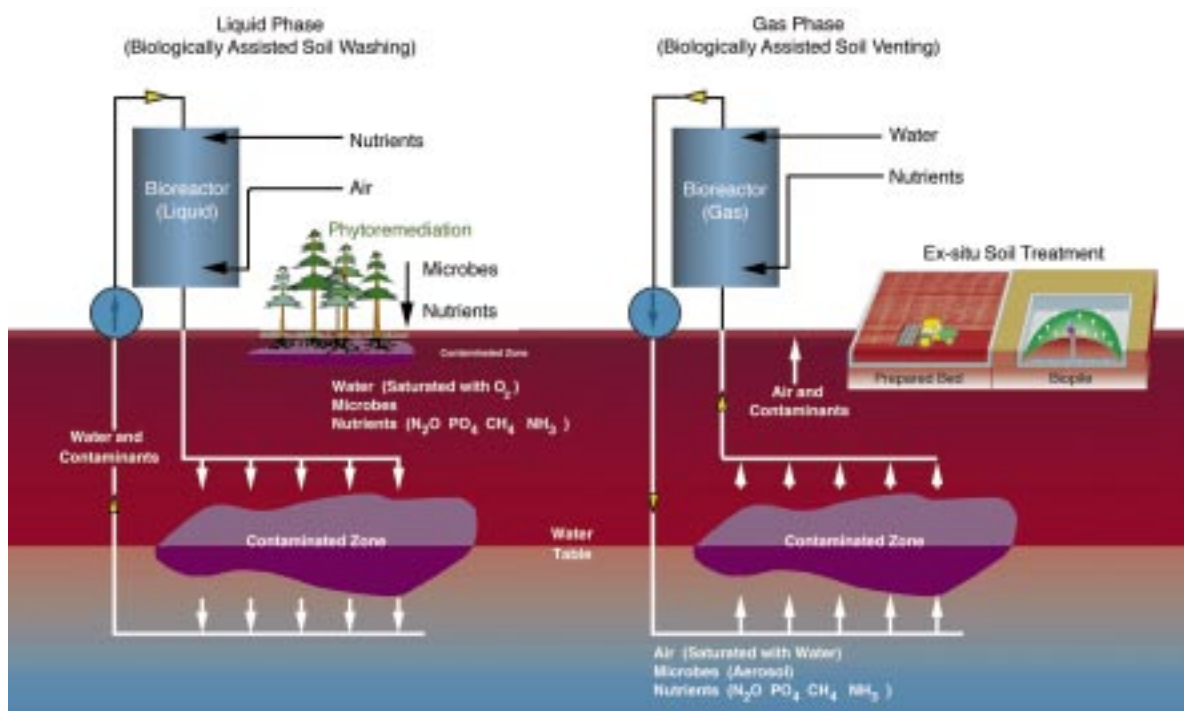


Figure 2.1. Bioremediation treatment strategies.

produced in on-site bioreactors may also be added to ex situ treatments such as engineered soil piles, or they may be injected into the subsurface for in situ treatment.

Ex situ bioaugmentation is a common technology at municipal wastewater treatment facilities. Commercial inoculants of enriched cultures consisting of one or more microbial species have been successfully used to colonize new trickling bed filter systems and to rapidly recolonize systems where the intrinsic microbial community was victim to a system upset.

Researchers are beginning to investigate genetically engineered microorganisms (GEMs) for use in bioaugmentation. Genetic engineering is the manipulation of genes to enhance the metabolic capabilities of an organism. In situ bioaugmentation with GEMs is still in the preliminary testing phase in fully or partly contained systems. There is a great deal of interest in the in situ use of GEMs for the treatment of hazardous wastes. Successful agricultural field trials with GEMs (e.g., nitrogen-fixing bacteria) and genetically altered plants (e.g., herbicide-resistant soybeans) are widespread. Organisms with enhanced capabilities to degrade hydrocarbons, aromatic compounds, and halogenated compounds have already been developed.

Theoretically, organisms could also be developed to degrade or transform heretofore recalcitrant compounds such as those containing metals and radionuclides. However, the application of genetic engineering technology for use in the environment remains controversial. This is partly due to the concern that GEMs are not “natural” and may persist in the environment, potentially causing an environmental upset like the rabbit introduction to Australia. Yet, the use of GEMs may be warranted when they are the only microorganisms that can transform or degrade a particularly hazardous contaminant.

Through its Bioremediation and Its Societal Implications and Concerns (BASIC) program, DOE addresses questions and concerns surrounding the field testing and release of microorganisms for environmental cleanup. This is being accomplished through communication and collaboration with all relevant stakeholders — community leaders and representatives, engineers, scientists, and lawyers, to name a few.

Intrinsic Bioremediation

Intrinsic bioremediation occurs in situ and relies on the already-existing naturally occurring biological processes. It is also known as natural attenuation. Intrinsic bioremediation was first noticed a number of years ago at sites of petroleum hydrocarbon contamination. The pollutants were being biodegraded by the naturally occurring microorganisms at rates fast enough to stop or reduce contaminant spread. In order to establish that intrinsic bioremediation is actually occurring at these rates, plume size and metabolic activity must be measured over a period of time.

At present, intrinsic bioremediation is mainly accepted for petroleum hydrocarbons and to a limited degree chlorinated hydrocarbons such as TCE. However, promising results have been obtained with intrinsic bioremediation of selenium-polluted agricultural drainage water in marshlands. Also, it is possible that scientists could take advantage of rapidly developing information on microbial processes in the subsurface, such as iron and sulfur reduction, to assess and perhaps reduce the need for the application of more costly and disruptive accelerated bioremediation technology.

Phytoremediation

Phytoremediation is the use of plants to remediate contaminated soils in the rhizosphere, which is the soil that surrounds and is influenced by plant roots and their associated microbial communities. Two forms of phytoremediation are phytoextraction and rhizofiltration. Phytoextraction is the use of metal-accumulating plants to remove toxic metals from soil. Rhizofiltration is the use of plant roots to remove toxic metals and radionuclides from contaminated waters. The plant root system serves both as a means for effective soil colonization and as a ready source of nutrients, with the result that microbial activity in the rhizosphere is greater and more easily sustained than in nonrhizosphere soil. In addition to uptake and transformation of organic compounds, many plants bioaccumulate metals and radionuclides. Hyperaccumulation of heavy metals (greater than 1% of dry weight) is common for plants that are acclimated to soils with high concentrations of cobalt, copper, chromium, lead, nickel, and zinc.

Phytoremediation technology has several advantages. It is inexpensive compared to conven-

tional technology and should prove cost effective for soils in which near-surface contamination is dispersed over broad areas.

Landfarming, Soil Piles, and Composting

Landfarming is the mixing of waste with surface soil over a tract of land. This technique has been used extensively to treat sludges from domestic sewage and industrial processes. The wastes are applied to soil surfaces as sludges or aqueous slurries, and the mixture is aerated through tilling. Optimal soil-water content is maintained and supplemental inorganic nutrients (N-P-K) added to stimulate microbial growth. Supplemental microorganisms may also be added. Although land farming has been an efficient and cost-effective means for treating a variety of wastes, adverse environmental effects sometimes have resulted, and this original landfarming method has been largely discontinued in the United States. A modified form of land farming has been adopted to comply with revised environmental regulations.

This modified form consists of sediment biopiles, or prepared beds, constructed above ground within contained treatment cells. This allows control of volatilization, leaching, and runoff. A vapor control system is constructed to ensure that volatile organic compounds (VOCs) are captured or destroyed. Current methods include adsorption to activated carbon for VOC disposal or destruction offsite.

Composting is a process applied to soil sediment biopiles that controls and utilizes heat generated by aerobic microbial metabolism. The material being composted serves as a source of nutrition for the microbes. Bulking agents, such as wood chips or straw, are often added to enhance air movement through a pile. This self-contained system generates and retains heat, eventually

raising the temperature of the compost pile. Composting has been used to biotransform explosives and propellants, in which the sediment piles are amended with manure or molasses to supply additional organic nutrients and microorganisms.

Land farming, prepared beds, biopiles, and composting hold a number of possibilities for bioremediation of radionuclides and metals by degrading organic chelating agents, altering pH, redox potentials, and production of biosurfactants. Any of these processes could be used to either mobilize, immobilize, or biotransform radionuclides and metals.

Slurries and Sediment Washing

Slurry bioreactors and sediment-washing equipment are commonly used to treat excavated sediment to which water is added. Slurry bioreactors are stirred tanks within which biodegradation or biotransformation takes place in an aerated environment. Sediment washing, which can be used in conjunction with the slurry process, is primarily a means of reducing the volume of contaminated sediment by solubilizing readily desorbed contaminants. It can be performed with or without accompanying biological treatment. Through rinsing, excavated sediments are screened to remove large debris, such as pipes, bricks, and concrete. Screened sediments are further divided by size into readily treatable material, such as sand and fine gravel, and silt-sized and colloidal material known as fines. The fines can be stored as contaminant waste or biotreated in a slurry reactor. The solubilized contaminants may be biodegraded or biotransformed in the initial washing or, alternatively, the now-contaminated wash water can be passed to a second reactor where biodegradation or biotransformation takes place.

CONTAMINANT PLUMES: MIGRATION OF HAZARDOUS WASTE IN THE SUBSURFACE

Contaminant plumes are zones of pollution extending downstream from sources of contamination. Contaminant types can vary in their rate of movement and distribution. Therefore, if more than one contaminant type has been released into the subsurface, multiple plumes can form with different distributions. Although a contaminated site can have a number of plumes with different contaminants or contaminant combinations, here we will examine the characteristics of a single “composite” contaminant plume (see Figure 2.2).

A source of contamination may be a single-point source such as a leaking tank. Or, the plume may have spread out from contamination of a large area, such as nitrate contamination of a water source caused by the general use of fertilizer on farm land. Point sources are frequently spills, treatment lagoons, and disposal sites such as cribs, trenches, landfills, and underground storage tanks.

Once a contaminant is released into the environment, the plume can spread into soils, unconsolidated sediments, rock formations, groundwater, and surface water. The contaminant itself may be in gaseous, liquid, or solid form, or a combination. Depending on the geology and hydrologic conditions at the site and the solubility of the contaminant, the plume may stay close to the source or be transported long distances by groundwater or rainwater infiltration events.¹ In some cases all of the contamination is caused by a single spill or leak. In others, the source of contamination may continue for decades — such as at an active waste disposal site — or when natural infiltration by rainwater or other surface water percolates down through the zone of contamination.

In the groundwater, the shape of a plume will depend on the rate of migration, which is largely controlled by groundwater flow directions and velocity, the geologic setting, the physical and chemical characteristics of the contaminant, and the presence of a continuing source. If the source has been stopped, the entire plume may migrate away from the original location, eventually becoming less concentrated through the transport processes of advection, diffusion, and dispersion, as well as chemical and biological reactions. These factors are briefly described below.

Advection is the transport of dissolved solutes with the bulk flow of water in the vadose zone (above the water table) or in groundwater. For highly soluble contaminants that do not undergo chemical or biological reactions with the geologic materials, advection is the primary mechanism influencing the fate and migration of the contaminant. Diffusion is the bulk movement of solutes resulting from thermally driven molecular motion of solutes. Through this random molecular motion, contaminants move from areas of high concentration to areas of lower concentration. Diffusion is thought to be particularly important when a geologic formation has a very low permeability or is very heterogeneous, such as a layered sequence of sand and clay. Dispersion is the mechanical mixing of solutes that occurs as the solutes are advected through the groundwater system.

1. Geohydrology and biogeochemistry in the vadose zone (unsaturated zone above the water table) are particularly important to DOE since some high-level radioactive waste (HLW) storage tanks at DOE sites have leaked over the last 40+ years. The leaks have been sporadic, and the composition of the waste in the HLW tanks has changed over the years. The pH of the solution in the tanks (>12), the temperature (>90°C), the presence of complex organics, the presence of multiple radionuclides with different valences and solubilities, and pumping activities in the tank can have extreme effects on the mobility and transport of contaminants and the activity of microorganisms in the vadose zone. Thus, the waste and waste-site activities can also have an extreme influence on the heterogeneity of contaminant plumes in the vadose zone.

Biological and chemical reactions also affect the size and shape of the plume — mostly by slowing or preventing migration of the contaminant. If the contaminants adsorb onto the geological materials, the rate of plume movement will be retarded (relative to the rate that water itself moves). Sometimes, however, contaminants adsorb onto very small particles, called colloids, that may themselves move with groundwater flow, thereby transporting the contaminant. Studies in the DOE's Subsurface Science program showed that both colloids and microorganisms accumulate at air–water interfaces.

In some cases, higher densities of microbes and higher concentrations of contaminants are observed at air–water interfaces, especially

capillary fringe zones in the vadose zone immediately above the water table. Water table fluxes can thereby cause unexpected concentration phenomena. Chemical and biological interactions also can result in precipitation of the contaminant into a solid phase that is no longer mobile. Organic contaminants also can be degraded into simpler molecules. Some of these are no longer toxic, but in some cases the so-called daughter product may be more toxic. Radioactive contaminants will spontaneously decay into their daughter products, which will have their own set of transport properties and reactivities. These decay products may form solid-, liquid-, or vapor-phase contamination products of their own, which must be factored into any remediation strategy.

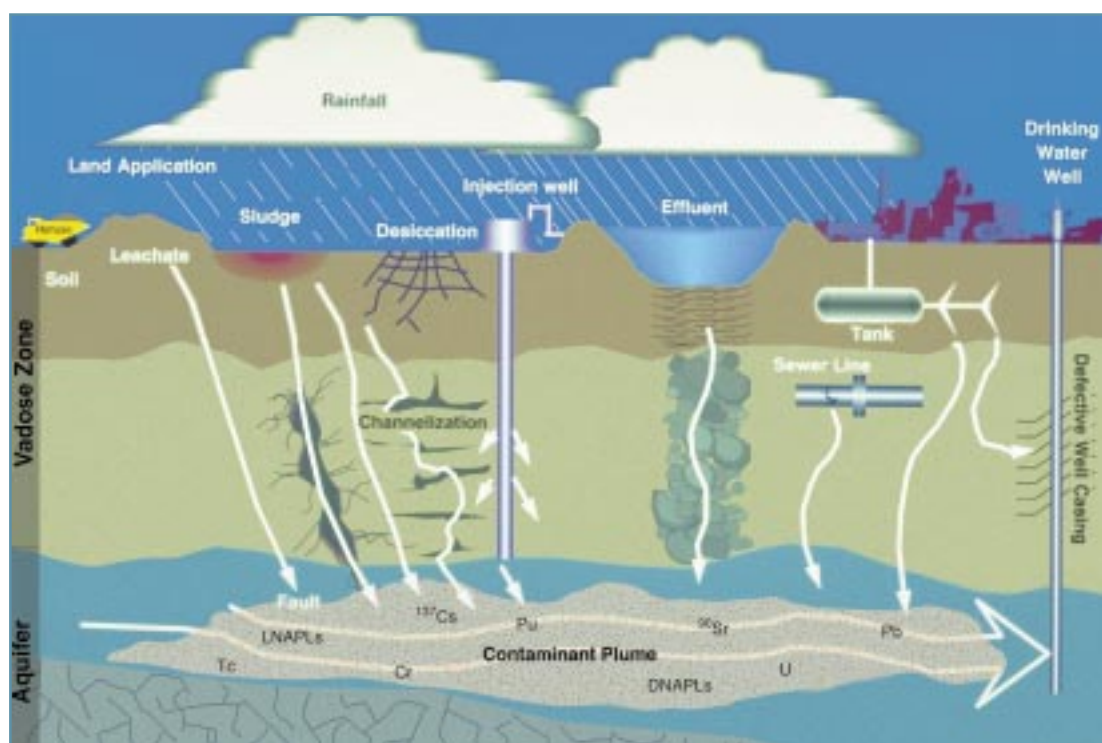


Figure 2.2. Sample contaminant plume consisting of mixed waste resulting from percolation from leaky tanks, landfills, basins, and trenches, as well as being formed through direct injection.